Experimental Parasitology 131 (2012) 111–115

Contents lists available at SciVerse ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Fatty acids oxidation and alternative energy sources detected in *Taenia crassiceps* cysticerci after host treatment with antihelminthic drugs

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ARTICLE INFO

Article history:
Received 27 September 2011
Received in revised form 27 February 2012
Accepted 15 March 2012
Available online 23 March 2012

Keywords:
Taenia crassiceps
Fatty acids oxidation
Alternative energy sources
Host treatment
Antihelminthic drugs

ABSTRACT

Human cysticercosis caused by *Taenia crassiceps* is rare however it is considered of zoonotic risk. The treatment of the infected patients was successful when using albendazole or praziquantel. The active forms of albendazole inhibit the glucose uptake and the active forms of praziquantel alter glycogen levels and nutrients absorption. The aim of this study was to analyze the production of organic acids that indicate the oxidation of fatty acids and the use of alternative energy sources from *T. crassiceps* cysticerci removed from the peritoneal cavity of mice treated with low dosages of albendazole (5.75 and 11.5 mg/kg) or praziquantel (3.83 and 7.67 mg/kg). The beta-hydroxibutyrate production was higher by the larval stage cysticerci in all treated groups and the propionate production was higher in final stage cysticerci treated with 11.5 mg/kg of albendazole when compared to the control group. The larval stages of cysticerci from the groups treated with 5.75 mg/kg of albendazole and 3.83 mg/kg of praziquantel produced more urea than the initial and final stages which indicate amino acids breakdown. We conclude that it was possible to detect the fatty acid oxidation and amino acids breakdown which indicate the use of alternative energy production sources as the used dosages only cause a partial blockage of the glucose uptake and leads to metabolic alterations in the cysticerci. The metabolic behavior observed after host treatment was different from former descriptions of the *in vitro* one which indicates great host-parasite interaction.

1. Introduction

In some regions of the world Taeniidae parasites still remain public health issues (Hoberg et al., 2000). *Taenia crassiceps* has wild canids as definitive host and may infect domestic ones, and rodents as intermediate hosts such as rats and marmots (Saeed et al., 2006; Bagrade et al., 2009; Stien et al., 2010; Willms and Zurabian, 2010). Human cysticercosis caused by *T. crassiceps* is rare however it constitutes a zoonotic risk especially to immunocompromised individuals accordingly to reports from France, Germany and England (Wünschmann et al., 2003). Cysticerci were detected in skeleton muscles, central nervous system, intra-ocular and subcutaneous regions. The treatment of these patients was successful when albendazole and praziquantel were used (Shea et al., 1973; Arocker-Mettinger et al., 1992; Klinker et al., 1992; Chermette et al., 1995; François et al., 1998; Maillard et al., 1998; Heldwein et al., 2006).

Albendazole is a drug from the benzimidazole group. Its active forms are effective when the host is treated and after *in vitro* exposure of adult forms, eggs and larvae of nematodes and cestodes (Horton, 2000; Marques et al., 2002). In those parasites the mode of action of this drug consists of the inhibition of the polymerization of beta-tubulin leading to inadequate levels of energy which are inconsistent to the helminth survival resulting in paralysis and death (Horton, 2000; Palomares et al., 2006). Praziquantel is considered the drug of choice in treatment campaigns due to its low cost and its pharmacologic properties against several parasites including *Schistosoma mansoni* and *Taenia solium* (Cioli et al., 1995; Cioli and Pica-Mattoccia, 2003; Doenhoff et al., 2009; Cederberg et al., 2012). Its active metabolites lead to muscle contractions, tegument injuries, alter the muscle glycogen levels and influence the nutrient absorption (Cioli et al., 1995; Cioli and Pica-Mattoccia, 2003; Jeziorski and Greenberg, 2006; Palomares et al., 2006).

The energetic metabolism from helminths in adult and larval forms differ from the host metabolism showing a great variety of nutrient catabolism pathways and the use of carbohydrates, fatty acids and proteins as energy production sources (Kita et al., 1997). In contrast with free living organisms parasites are nutritionally dependent from their hosts. The variety of habitats during its life cycle induces complex nutritional and biochemical adaptations to insure the parasite survival (Köhler and Voigt, 1988).

There are *in vitro* and *in vivo* reports on the diffusion mechanisms and mode of action of antihelminthic drugs such as

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0014-4894
http://dx.doi.org/10.1016/j.exppara.2012.03.017

albendazole and praziquantel (Alvarez et al., 2007; Vinaud et al., 2008, 2009), as well as reports of ultra structural injuries (Palomares et al., 2004, 2006; Palomares-Alonso et al., 2007) and structural ones in *T. crassiceps* cysticerci (Venkatesan, 1998; Cioli et al., 1995; Cioli and Pica-Mattoccia, 2003; Horton, 2000) related to exposure to these drugs. There are also *in vitro* reports that evaluate the excretion/secretion of organic acids related to metabolic alterations of glycolytic pathways and the tricarboxylic acid cycle after the exposure to these drugs (Vinaud et al., 2007, 2008) and the detection of energy production from alternative energy sources such as fatty acid oxidation (Vinaud et al., 2009).

However there is not a report of the use of these alternative energy sources by *T. crassiceps* cysticerci after the host treatment. Therefore the aim of this study was to analyze the production of organic acids that indicate the oxidation of fatty acids such as acetate, acetoacetate, beta-hydroxybutirate and propionate, and evaluate the use of alternative energy sources such as amino acids breakdown through urea and creatinine dosages in *T. crassiceps* cysticerci after the host treatment with low dosages of albendazole or praziquantel.

2. Materials and methods

2.1. Maintenance of the T. crassiceps biological cycle

The biological cycle of *T. crassiceps* (ORF strain) has been maintained in the vivarium of the Tropical Pathology and Public Health Institute from the Federal University of Goias (IPTSP/UFG) since 2002. Ten initial phase cysticerci were inoculated in the intraperitoneal cavity of 8–12 week old female BALB/c mice where they multiplied by budding. Approximately 90 days after inoculation the animals were euthanized and necropsied. The cysticerci were removed from the intraperitoneal cavity, 10 initial phase specimens were selected (Vinaud et al., 2008) and inoculated in other non-infected mice for cycle continuation (Espíndola et al., 2002; Vaz et al., 1997).

The ethical principles for animal experimentation professed by the Brazilian society of laboratory animal sciences (Sociedade Brasileira de Ciência em Animais de Laboratório/SBCAL) were followed and this study was authorized by the Committee for Ethical Research of the Federal University of Goias (CoEp/UFG) (registration number 008/09).

The mice received daily care, acidified water and standard rations.

2.2. Mice infection and treatment

The BALB/c female mice were intraperitoneally inoculated with 10 initial phase *T. crassiceps* cysticerci (Vinaud et al., 2008), using 1 mL syringes. Thirty days after infection they were gavage treated with low single doses of praziquantel (Merck®) and albendazole (Vitapan®), 24 h after treatment they were euthanized for better observation of the biochemical effects of the drugs on the cysticerci.

The infected mice were divided into four groups, namely, group A: consisting of five infected mice treated with a single dose of 5.75 mg/kg of albendazole; group B: consisting of five infected mice treated with a single dose of 11.5 mg/kg of albendazole; group C: consisting of five infected mice treated with a single dose of 3.83 mg/kg of praziquantel; group D: consisting of five infected mice treated with a single dose of 7.67 mg/kg of praziquantel. A control group was formed consisting of five infected mice that did not receive treatment.

All the experiment was performed in quintuplicate. The doses applied were determined according to the manufacturer's recommendation and the recommended to cysticercosis treatment (Matthaiou et al., 2008) and then reduced to a level lower than necessary to eliminate the parasite, as the purpose of the study was to access the adaptability of the parasite to the drugs.

2.3. Cysticerci biochemical analysis

The cysticerci removed from the mice were classified macroscopically according to their evolutionary phase as initial, larval and final (Vinaud et al., 2007). The specimens were fixed in liquid nitrogen, homogenized with 12% perchloric acid as described by Vinaud et al. (2007), and then the organic acids were extracted for chromatographic analysis as described by Bezerra et al. (1999) and Vinaud et al. (2007, 2008).

The organic acids were identified through high performance liquid chromatography (HPLC) according to the previously determined retention time and calibration. The organic acids analyzed were the ones which indicate the fatty acids metabolism such as acetate, acetoacetate, beta-hydroxybutirate and propionate (Bezerra et al., 1999; Vinaud et al., 2007, 2008).

After the samples were homogenized with 12% percloric acid they were centrifuged at 500 g/10 min and from the supernatant the urea and creatinine dosages were performed by dose response spectrophotometry using a KoneLab 60i apparatus, according to the commercial kit protocol, enzymatic method, Wiener lab®.

2.4. Statistical analysis

The statistical analysis was performed using the Sigma Stat 2.3 program. Descriptive statistics were applied to determine the mean and standard deviation and to evaluate the differences between the groups analyzed. The variables were tested for normal distribution and homogeneous variance. As they presented normal distribution, variance analysis was used. The differences noted were considered significant when $p < 0.05$.

3. Results and discussion

This study analyzed the use of alternative sources of energy production by *T. crassiceps* cysticerci after the host treatment with low dosages of antihelminthic drugs that impair the glucose uptake and the traditional energy production pathways. This study detected the adaptation of the parasite when facing a hostile environment inside its host.

It was possible to detect in cysticerci removed from mice treated with 5.75 and 11.5 mg/kg of albendazole and 3.83 to 7.67 mg/kg of praziquantel the production of organic acids that indicate the oxidation of fatty acids such as acetoacetate, beta-hydoxybutirate and propionate (Tables 1 and 2).

It was not possible to detect acetate in the analyzed samples. We believe that this occurred similarly to what occurs in mammals as the acetate was combined with acetyl-CoA originating acetoacetyl-CoA which is combined with acetyl-CoA forming beta-hydroxy-beta-methylglutaryl-CoA which is used to form acetoacetate which may be excreted (Lehninger et al., 2006). In accordance to the description made by Vinaud et al. (2009) in *in vitro* studies which did not detect any difference between the production of acetate from *T. crassiceps* cysticerci after exposure to the same drugs. Other studies with cestodes such as *Echinococcus granulosus* and *Hymenolepis diminuta* showed an increase in acetate production when there is excess of intramitochondrial pyruvate as the produced acetate is not converted into acetyl-CoA which is a substrate to the tricarboxylic acid cycle (Melhorn et al., 1988; Vinaud et al., 2009).

Table 1
Concentration (mean ± standard deviation) of products from *T. crassiceps* cysticerci from initial, larval and final phases after host treatment with albendazole.

	Control			ABDZ 5.75 mg/kg			ABDZ 11.5 mg/kg		
	Initial	Larval	Final	Initial	Larval	Final	Initial	Larval	Final
Urea (mg/dL)	2.40 ± 1.00	5.75 ± 3.5	2.00 ± 1.00	1.00 ± 1.00	3.00 ± 2.00*	1.20 ± 1.00	2.34 ± 1.00	3.44 ± 1.50	1.83 ± 1.00
Beta-hydroxybutirate (pMol/L)	2.37 ± 0.90	10.32 ± 6.28*	1.80 ± 0.82	3.07 ± 0.74	23.46 ± 3.26*	2.39 ± 0.43	1.38 ± 0.86	6.83 ± 3.26*	1.30 ± 0.56
Propionate (pMol/L)	1.79 ± 1.51	5.29 ± 1.21*	1.47 ± 0.99	2.44 ± 1.59	1.74 ± 1.31	1.88 ± 1.05	1.83 ± 1.14	1.58 ± 1.19	3.05 ± 1.71*

ABDZ – albendazole.
* $p < 0.05$.

Table 2
Concentration (mean ± standard deviation) of products from *Taenia crassiceps* cysticerci from initial, larval and final phases after host treatment with praziquantel.

	Control			PZQ 3.83 mg/kg			PZQ 7.67 mg/kg		
	Initial	Larval	Final	Initial	Larval	Final	Initial	Larval	Final
Urea(mg/dL)	2.40 ± 1.00	5.75 ± 3.50	2.00 ± 1.00	2.00 ± 1.00	3.81 ± 1.75*	1.40 ± 1.00	1.33 ± 1.00	8.44 ± 3.75	1.33 ± 1.00
Beta-hydroxybutirate(pMol/L)	2.37 ± 0.90	10.32 ± 6.28*	1.80 ± 0.82	1.55 ± 0.69	7.19 ± 2.50*	1.83 ± 0.83	2.54 ± 0.87	2.37 ± 0.90	10.32 ± 6.28*
Propionate (pMol/L)	1.79 ± 1.51	5.29 ± 1.21	1.47 ± 0.99	2.05 ± 1.36	1.84 ± 1.33	2.41 ± 1.39	1.79 ± 1.32	1.79 ± 1.51	5.29 ± 1.21

PZQ – praziquantel.
* $p < 0.05$.

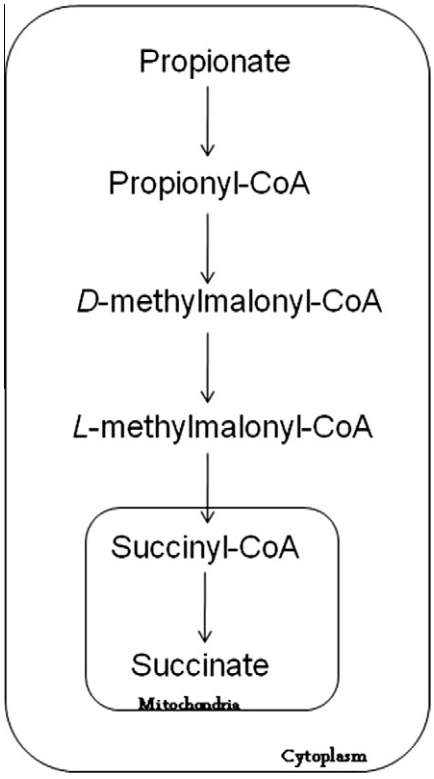

Fig. 1. Production of succinate from propionate (Adapted from Lehninger et al., 2006).

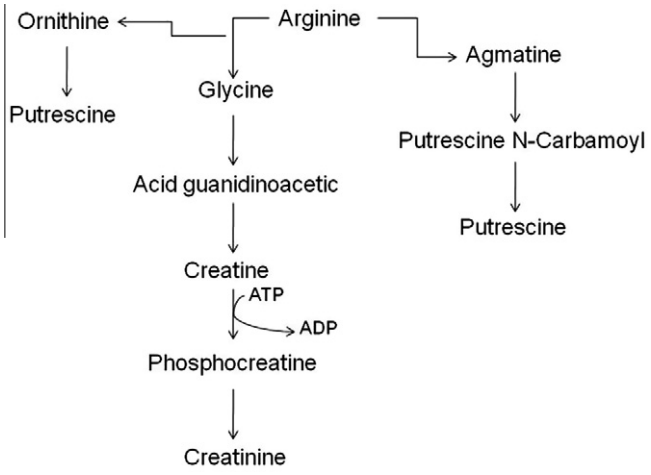

Fig. 2. Pathway of creatinine and putrescine production from arginine (Adapted from Metzler, 1977).

It was not possible to detect acetoacetate in any of the analyzed samples. This result is in accordance to the description by Vinaud et al. (2009) in *in vitro* studies. We believe that the acetoacetate was probably consumed by D-beta-hydroxybutirate dehydrogenase enzyme to produce beta-hydroxybutirate (Lehninger et al., 2006) similarly to what is observed in mammals.

The beta-hydroxybutirate production was greater by all larval stages of the treated groups because the mode of action of both drugs impair glucose uptake (Tables 1 and 2) (Venkatesan, 1998; Horton, 2000). Therefore there is the activation of alternative pathways of energy production such as the beta-oxidation of fatty acids similarly to what is observed in mammals (Lehninger et al., 2006) and to what is described by Vinaud et al. (2009) when studying this same parasite and detected an *in vitro* increase of the secretion/excretion of this product by the final stage cysticerci after exposure to the same drugs.

The propionate production was greater by the final stage treated with 11.5 mg/kg of albendazole when compared to the control group (Table 1). We believe that this occurred because the mode of action of this drug is to block the glucose uptake (Venkatesan, 1998; Horton, 2000) and therefore leading the parasite to use as an alternative energy source the oxidation of fatty acids which results in the increase of propionate. The higher concentrations of this product will lead to its conversion into succinate which may be consumed as substrate to the electrons transporting chain inside the mitochondrion as it is observed in mammals (Lehninger et al., 2006) (Fig 1).

The urea production was greater in cysticerci from the hosts treated with 5.75 mg/kg of albendazole (Table 1). The same was observed from the group which was treated with 3.83 mg/kg of praziquantel (Table 2). We believe that this occurred in those larval stages because there is a greater use of the carbonic chain from amino acids to the nucleic acids synthesis (Lehninger et al., 2006;

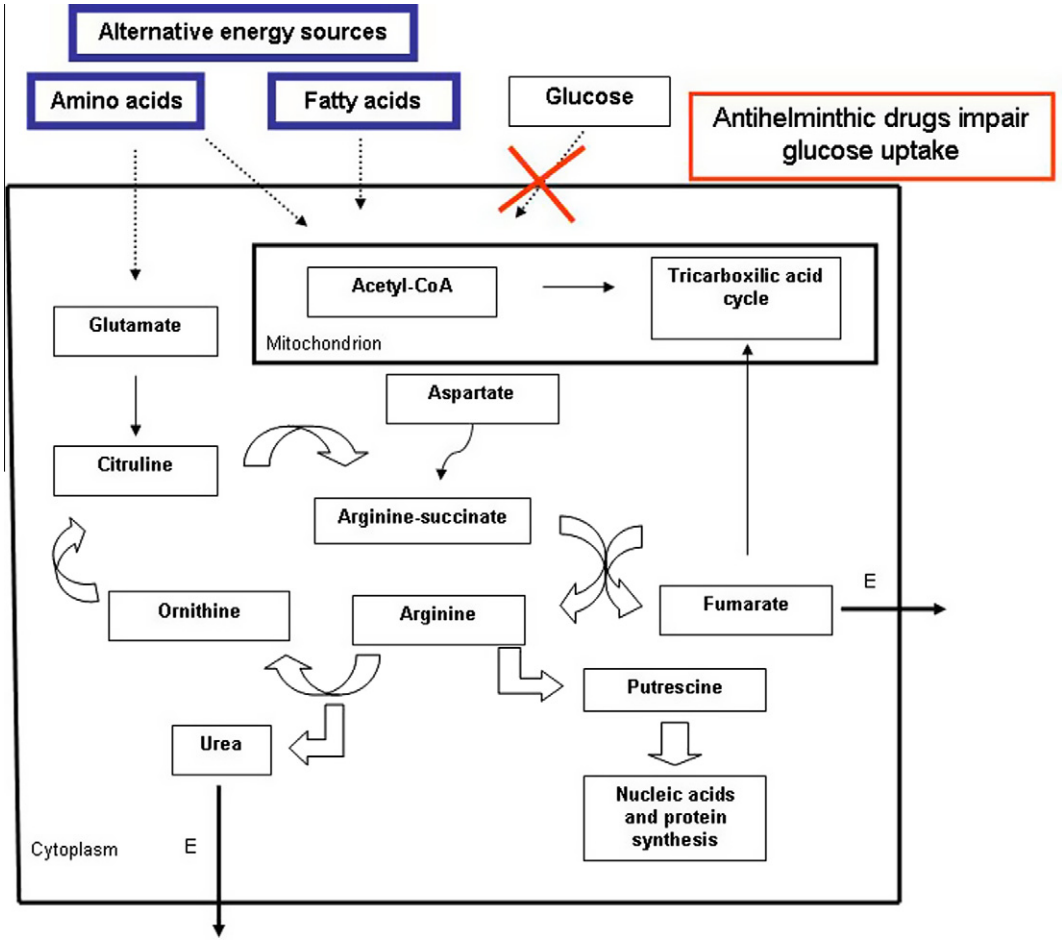

Fig. 3. Use of alternative energy sources by *Taenia crassiceps* cysticerci after host treatment with low dosages of antihelminthic drugs. The figure represents a single cell from the parasite. Amino acids catabolism and fatty acids oxidation results in urea excretion. E-products from the metabolism which are excreted.

Vinaud et al., 2009). The urea production is important to the parasite's survival because it is a pathway to the excretion of toxic substances as described in *Fasciola gigantica* (Mohamed et al., 2005) and in *T. crassiceps* cysticerci (Vinaud et al., 2009).

The creatinine production was not detected in any of the analyzed samples. We suggest that this occurred because one of the creatinine production pathways is from arginine (Fig 2) as the amidine terminal group from arginine is transferred to the formation of ornithine and consequently forming the guanidinoacetic acid which is transformed in creatinine and present as an intermediary product glycine. The creatine kinase enzyme using a phosphate group converts creatine into phosphocreatine which is an ATP source especially during muscle contractions in which the creatine kinase enzyme perform the hydrolysis of the ADP forming ATP and creatinine. The non detection of creatinine may be explained due to the use of arginine to poliamines production such as putrescine which has an important role in nucleic acids and protein synthesis besides the modulation of ion channels during the muscle contraction (Figs. 2 and 3) (Metzler, 1977).

In summary as the anti-helminthic drugs impair the glucose uptake there is an enhancement of the use of alternative energy sources such as the fatty acids oxidation and the amino acids breakdown. The increase of the latter pathway leads to greater urea excretion as it acts as a detoxification mechanism. Also the ornithine cycle produces fumarate which may be excreted, as described previously by our group (Fraga et al., 2012) or used in the TCA cycle as it may pass through the mitochondrion membrane. On the other hand the increase of the fatty acids oxidation leads to production of Acetyl-CoA which is used in the TCA cycle enabling the aerobic production of energy through the oxidative phosphorylation in the electron transport chain (Fig. 3).

In conclusion it was possible to detect the oxidation of fatty acids and the use of alternative energy sources in *T. crassiceps* cysticerci after the host treatment with low dosages of antihelminthic drugs. The used dosages were not capable of total blockage of the glucose uptake however it was enough to alter the parasite's metabolism and to induce the use of alternative energy sources such as fatty acids oxidation and amino acids catabolism. Therefore the cysticerci exposed to the drugs through host treatment presented metabolic adaptations different from the ones described after *in vitro* treatments which indicate a great host-parasite interaction.

Acknowledgments

We are grateful to Sharon Lois Vinaud for the English review of the manuscript. The authors would also like to thank CAPES (Coordenação de aperfeiçoamento de pessoal de nível superior), CNPq (Conselho nacional de desenvolvimento científico e tecnológico) and FAPEG (Fundação de apoio a pesquisa do estado de Goiás) for the financial support.

References

Alvarez, L.I., Mottier, M.L., Lanusse, C.E., 2007. Drug transfer into target helminth parasites. Trends Parasitol. 23 (3), 97–104.

Arocker-Mettinger, E., Huber-Spitzy, V., Auer, H., Grabner, G., Stur, M., 1992. *Taenia crassiceps* in the anterior chamber of the human eye: a case report. Klin. Monatsbl. Augenheilkd. 201 (1), 34–37.

Bagrade, G., Kirjusina, M., Vismanis, K., Ozolins, J., 2009. Helminth parasites of the wolf *Canis lupus* from Latvia. J. Helminthol. 83, 63–68.

Bezerra, J.C.B., Kemper, A., Becker, W., 1999. Profile of organic acid concentrations in the digestive gland and hemolynph of *Biomphalaria glabrata under estivation*. Mem. Inst. Oswaldo Cruz 94 (6), 779–784.

Cederberg, C., Sikasunge, C.S., Andersson, A., Johansen, M.V., 2012. Short communication: *in vitro* efficacy testing of praziquantel, ivermectin, and oxfendazole against *Taenia solium* cysts. J. Parasitol. Res. 2012, 363276.

Chermette, R., Bussieras, J., Marionneau, J., Boyer, E., Roubin, C., Prophette, B., Maillard, H., Fabiani, B., 1995. Invasive cysticercosis due to *Taenia crassiceps* in an AIDS patient. Bull. Acad. Natl. Med. 179 (4), 777–783.

Cioli, D., Pica-Mattoccia, L., 2003. Praziquantel. Parasitol. Res. 90, S3–S9.

Cioli, D., Pica-Mattoccia, L., Archer, S., 1995. Antischistosomal drugs: past, present and future? Pharmacol. Ther. 68 (1), 35–85.

Doenhoff, M.J., Hagan, P., Cioli, D., Southgate, V., Pica-Mattoccia, L., Botros, S., Coles, G., Tchuem Tchuente, L.A., Mbaye, A., Engels, D., 2009. Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs. Parasitology 136, 1825–1835.

Espíndola, N.M., Vaz, A.J., Pardini, A.X., Fernandes, I., 2002. Excretory/secretory antigens (ES) from *in vitro* cultures of *Taenia crassiceps* cysticerci, and use of an anti-ES monoclonal antibody for antigen detection in samples of cerebrospinal fluid from patients with neurocysticercosis. Ann. Trop. Med. Parasitol. 96 (4), 361–368.

Fraga, C.M., Costa, T.L., Bezerra, J.C., Lino-Junior, R.S., Vinaud, M.C., 2012. *Taenia crassiceps*: host treatment alters glycolisis and tricarboxilic acid cycle in cysticerci. Exp. Parasitol. 130 (2), 146–151.

François, A., Favennec, L.P., Cambon-Michot, C., Gueit, I., Biga, N., Tron, F., Brasseur, P., Hemet, J., 1998. *Taenia crassiceps* invasive cysticercosis: a new human pathogen in acquired immunodeficiency syndrome? Am. J. Surg. Pathol. 22 (4), 488–492.

Heldwein, K., Biedermann, H., Hamperi, W., Bretzel, G., Loscher, T., Laregina, D., Frosch, M., Büttner, D.W., Tappe, D., 2006. Subcutaneous *Taenia crassiceps* infection in a patient with non-Hodgkin's lymphoma. Am. J. Trop. Med. Hyg. 75 (1), 108–111.

Hoberg, E.P., Jones, A., Rausch, R.L., Emon, K.S., Gardner, S.L., 2000. A phylogenetic hypothesis for species of the genus *Taenia* (Eucestoda: Taeniidae). J. Parasitol. 86, 89–98.

Horton, J., 2000. Albendazole: a review of anthelmintic efficacy and safety in humans. Parasitology 121, S113–S132.

Jeziorski, M.C., Greenberg, R.M., 2006. Voltage-gated calcium channel subunits from platyhelminths: potential role in praziquantel action. Int. J. Parasitol. 36, 265–632.

Kita, K., Hirawake, H., Takamiya, S., 1997. Cytochromes in the respiratory chain of helminth mitochondria. Int. J. Parasitol. 27 (6), 617–630.

Klinker, H., Tintelnot, K., Joeres, R., Müller, J., Gross, U., Schmidt-Rotte, H., Landwehr, P., Richter, E., 1992. *Taenia crassiceps* infection in AIDS. Dtsch. Med. Wochenschr. 117, 133–138.

Köhler, P., Voigt, W.P., 1988. Nutrition and metabolism. In: Melhorn, H. (Ed.), Parasitology in Focus Facts and Trends. Springer-Verlag, Berlim, Alemanha, pp. 412–453.

Lehninger, A.L., Nelson, D.L., Cox, M.M., 2006. Princípios de Bioquímica, 4ª ed. Sarvier Editora de Livros Médicos Ltda, São Paulo, xxix + pp. 1202.

Maillard, H., Marionneau, J., Prophette, B., Boyer, E., Celerier, P., 1998. *Taenia crassiceps* cysticerci and AIDS. AIDS 12, 1551–1552.

Marques, M.P., Takayanagi, O.M., Lanchote, V.I., 2002. Albendazole metabolism in patients with neurocysticercosis: antipyrine as a multifunctional marker drug of cytochrome P450. Braz. J. Med. Biol. Res. 35, 261–269.

Matthaiou, D.K., Panos, G., Adamidi, E.S., Falagas, M.E., 2008. Albendazole versus praziquantel in the treatment of neurocysticercosis: a meta-analysis of comparative trials. PLOS Neglected Dis. 2 (3), e194.

Melhorn, H., Franz, M., Taraschewski, H., Voight, W.P., Walldorf, V., 1988. Morphology. In: Melhorn, H. (Ed.), Parasitology in Focus: Facts and Trends, 1ª ed. Springer-Verlag, Berlim, Alemanha, p. 924.

Metzler, D.E., 1977. The metabolism of nitrogen and amino acids. In: Metzler, D.E. (Ed.), Biochemistry The Chemical Reaction of Living Cells, vol. 2, 2ª ed. Elsevier Academic Press, Amsterdam, Holanda, pp. 358–1419.

Mohamed, S.A., Fahmy, A.S., Mohamed, T.M., Hamdy, S.M., 2005. Urea cycle of *Fasciola gigantica:* purification and characterization of arginase. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 142, 308–316.

Palomares, F., Palencia, G., Ambrosio, J.R., Ortiz, A., Cook, H.J., 2006. Evaluation fo the efficacy of albendazole sulphoxide and praziquantel in comination on *Taenia crassiceps: in vitro* studies. J. Antimicrob. Chemother. 57, 482–488.

Palomares, F., Palencia, G., Pérez, R., Gonzáles-Esquivel, D., Castro, N., Cook, H.J., 2004. *In vitro* effects of albendazole sulphoxide and praziquantel against *Taenia solium* and *Taenia crassiceps* cysts. J. Antimicrob. Chemother. 48 (6), 2302–2304.

Palomares-Alonso, F., Piliado, J.C., Palencia, G., Ortiz-Prata, A., Cook, H.J., 2007. Efficacy of nitazoxanide, tizoxanide and tizoxanide/albendazole sulphoxide combination against *Taenia crassiceps* cysts. J. Antimicrob. Chemother. 59, 212–218.

Saeed, I., Maddox-Hyttel, C., Monrad, J., Kapel, C.M.O., 2006. Helminths of red foxes *(Vulpes vulpes)* in Denmark. Vet. Parasitol. 139 (1–3), 168–179.

Shea, M., Maberley, A.L., Walers, J., Freeman, R.S., Fallis, A.M., 1973. Intraocular *Taenia crassiceps* (Cestoda). Trans. Am. Acad. Ophthalmol. Otolaryngol. 77, OP778–OP783.

Stien, A., Voutilaine, L., Hausisalmi, V., Fuglei, W., Mork, T., Yoccoz, N.G., Ims, R.A., Henttonen, H., 2010. Intestinal parasites of the Arctic fox in relation to the abundance and distribution of intermediate hosts. Parasitology 137 (1), 149–157.

Vaz, A.J., Numes, C.M., Piazza, R.M., Livramento, J.A., Da Silva, M.V., Nakamura, P.M., 1997. Immunoblot with cerebrospinal fluid form patients with neurocysticercosis using antigen from cysticerci of *Taenia solium* and *Taenia crassiceps*. Am. J. Trop. Med. Hyg. 57, 354–357.

Venkatesan, V., 1998. Albendazole. J. Antimicrob. Chemother. 41, 145–147.

Vinaud, M.C., Ferreira, C.S., Lino Junior, R.S., Bezerra, J.C.B., 2008. *Taenia crassiceps*: energetic and respiratory metabolism from cysticerci exposed to praziquantel and albendazole *in vitro*. Exp. Parasitol. 120, 221–226.

Vinaud, M.C., Ferreira, C.S., Lino Junior, R.S., Bezerra, J.C.B., 2009. *Taenia crassiceps:* fatty acids oxidation and alternative energy source in *in vitro* cysticerci exposed to antihelminthic drugs. Exp. Parasitol. 122 (2), 208–211.

Vinaud, M.C., Lino Junior, R.S., Bezerra, J.C.B., 2007. *Taenia crassiceps* organic acids detect in cysticerci. Exp. Parasitol. 116, 335–339.

Willms, K., Zurabian, R., 2010. *Taenia crassiceps: in vivo* and *in vitro* models. Parasitology 137 (3), 335–346.

Wünschmann, A., Garlie, V., Averbeck, G., Kurtz, H., Hoberg, E.P., 2003. Cerebral cysticercosis by *Taenia crassiceps* in a domestic cat. J. Vet. Diagn. Invest. 15, 484–488.